bation with plasma for 2 hr at 37° C,[1] the pH of the digestion mixture being 4.5.

Striking results were obtained with a crude extract of baker's yeast (containing 3.7 μg B_c per cc in conjugated form) from which 0.2 cc plasma liberated 0.7 μg folic acid in 1 hr at 37° C.

Additional evidence of the enzymatic role of plasma is offered by the use, as substrate, of pure crystalline vitamin B_c conjugate,[2] which gives identical results.

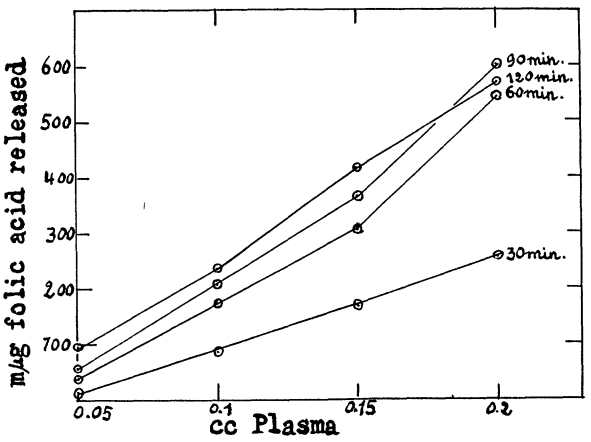

FIG. 1. Liberation of folic acid after various incubation periods at 37° C of 0.5 cc yeast extract + human plasma + 5 cc sodium acetate m/10 at pH 4.5 + water (total volume = 10 cc).

The B_c release as a function of plasma concentration for a constant time of incubation is represented in Fig. 1. There appears to be a linear relationship for plasma concentrations, ranging from 0.05 cc to 0.15 cc.

The study of human plasma conjugase activity in normal and pathological conditions has given the following results: under standard conditions, viz., with incubation at 37° C and pH 4.5, a mixture of 0.15 cc heparinized plasma and 0.5 cc yeast extract made up to a volume of 10 cc, liberated 0.5 μg to 1.5 μg folic acid per cc plasma in 90 min, with a maximum frequency of 0.8 μg to 1.0 μg. No significant variation of conjugase activity could be detected in pernicious anemia or in other pathological conditions, with the exception of asystolic patients, where the amount of conjugase per cc plasma was sometimes considerably decreased and attained values of 0.2 μg to 0.7 μg folic acid.

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[1] In a recent communication published while this article was in press, Simpson and Schweigert came to similar conclusions. (*Arch of Biochem.,* 1949, **20**, 32.)

[2] Crystalline vitamin B_c conjugate used in this experiment was supplied by Parke, Davis & Co. Laboratories, Detroit.

The Effects of Choline and Methionine on Phospholipide Formation in Patients with Liver Disease as Measured by Radioactive Phosphorus[1]

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In the past ten years the importance of nutritional factors in the pathogenesis and prophylaxis of experimentally induced cirrhosis in animals has resulted in a modification of the therapeutic regime in human patients. Of special interest has been the use of a diet high in protein and the administration of vitamin supplements, liver extracts, and lipotropic factors, such as methionine and choline. The efficacy of the above regimen is reflected in the lower mortality figures now being reported in the treatment of advanced instances of cirrhosis (*3*). In general this improvement is ascribed in part to a regenerative action on the liver tissue and in part to the lipotropic action of methionine and choline, resulting in the removal of the fat accumulated in the liver. The latter effect has been interpreted as being due to an increased formation of phospholipides.

In experimental animals with dietary fatty liver it has been shown, by the use of radioactive phosphorus as an indicator, that the administration of choline (*2*) or methionine (*8*) causes an increase in the rate of the synthesis of phospholipides in the liver. Since the plasma phospholipides are synthesized almost entirely in the liver (*5*), it seems that changes in the rate of formation of liver phospholipides would be reflected by corresponding changes in the amounts of newly formed phospholipides in the plasma. Indeed, in dogs on a diet low in protein and high in fat (*7*) a stimulatory effect by choline on the turnover of phospholipides in plasma has been observed.

We now have in progress a systematic study of the turnover of plasma phospholipides in normal human beings and in patients with various diseases. Some preliminary findings have been reported in a summarized form (*4*) and more extensive data will be published elsewhere. We are here reporting only some results obtained in two cirrhotic patients who exhibited a marked response to a single large dose of methionine or choline. These results suggest certain possibilities which might be of considerable interest in the interpretation and treatment of this disease.

One 31-year-old white male (O.A.) and one 36-year-old

[1] Supported in part by a grant from the U. S. Public Health Service. The P^{32} used in these experiments was obtained from the National Laboratories, Oak Ridge, Tennessee on allocation from the U. S. Atomic Energy Commission.

[2] We wish to acknowledge the helpful suggestions and constructive criticism of Dr. Camillo Artom and the technical assistance of Dr. Benjamin Vatz and Mr. Jesse C. Trott, Jr.

white female (N.M.H.) having clinical evidence of advanced portal cirrhosis were studied. At the time of admission, both were jaundiced and had demonstrable ascites, edema, and hepatic enlargement. Both gave a history of marked alcoholism. Definite impairment of liver function was found, as demonstrated by determinations of total serum proteins, albumin and globulin, vitamin A levels and by various tests, such as bromsulphalein,

TABLE 1

EFFECT OF LIPOTROPIC AGENTS ON SPECIFIC ACTIVITY OF PLASMA PHOSPHOLIPIDES

Patient	Treatment	Time in hr	Plasma phospholipides: Radioactivity per 100 cc* r.r.u.	Plasma phospholipides: Mg phosphorus per 100 cc†	Plasma phospholipides: Specific activity‡ × 100
O.A.	Injection of P^{32} + 9g choline orally	0	....	11.70	...
		24	1.785	11.16	16.0
		48	1.740	10.25	17.0
	P^{32}	0	....	7.96	...
		24	0.450	6.96	6.4
		48	0.766	6.23	12.3
		84	1.356	8.92	15.1
	P^{32} + 9g choline orally	0	....	11.25	...
		24	0.710	11.72	6.1
		48	1.012	11.36	8.9
N.M.H.	P^{32} + 6g methionine I.V.	0	....	11.84	...
		24	3.810	11.10	34.3
	P^{32}	0	....	9.01	...
		24	0.531	6.41	8.3
		48	1.270	7.60	16.7
		72	1.770	9.40	18.8
	P^{32} + 10g methionine I.V.	0	....	5.11	...
		24	0.712	6.70	10.6
		48	1.334	8.28	16.1
		72	1.700	9.68	17.5

* Expressed in relative radioactivity units (r.r.u.), the total dose injected being equal to 10^4 r.r.u.

† From the lipide P the total plasma phospholipide can be calculated (mg of P × 22.7).

‡ The specific activity is the ratio of the radioactivity (in r.r.u.) to the phosphorus (in mg) in the lipide extracts.

galactose tolerance, and hippuric acid excretion. In both instances, needle aspiration biopsy of the liver showed cirrhosis and fatty infiltration.

The patients were given an intramuscular injection of radiophosphorus in the form of Na_2HPO_4 (0.5 mc). Blood samples were taken routinely at 0, 24, 48, and 72 (or 96) hr. The plasma was separated and the lipids extracted with hot alcohol, alcohol-ether, and chloroform. On aliquots of the chloroform solution, the radioactivity (*1, 2*) and the phosphorus (*6*) were determined. At the time of the first injection of the radiophosphorus, the patients were given a single large dose of methionine (6 g intravenously to patient N.M.H.), and choline chloride (9 g orally to patient O.A.). After several months of treatment with methionine (1 g three times per day), the patients were reinjected with radiophosphorus (without simultaneous administration of the lipotropic agents) and the determinations were repeated on the plasma lipids. Two months later, a third experiment was made, in which

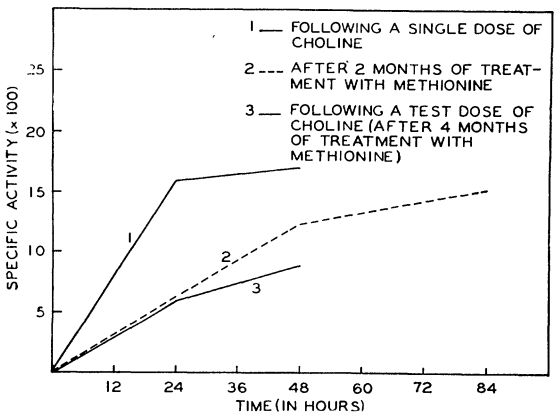

FIG. 1. Phospholipide turnover in a 31-year-old man with cirrhosis.

the effect of a single large dose of the substances was again studied, as in the first experiment.

The results are shown in Figs. 1 and 2 and Table 1. In the two cirrhotic patients previously untreated, the specific activity values of the plasma lipids following the

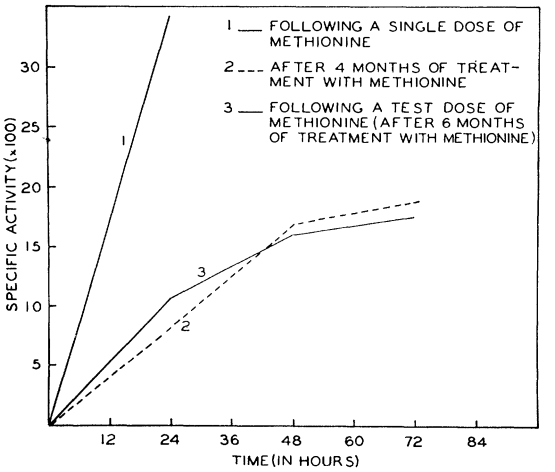

FIG. 2. Phospholipide turnover in a 36-year-old woman with cirrhosis.

initial large dose of methionine or choline were considerably higher than the corresponding values found in the same patients in later periods, with or without administration of the massive dose of lipotropic substances. It seems logical to conclude that in these patients the initial large dose of the lipotropic substances actually caused a marked increase in the rate of phospholipide turnover and that such an increase was no longer obtainable after prolonged therapy with methionine. This last finding is in keeping with similar results we have obtained in a number of other patients who were also treated with

methionine over a long period of time (*4*). In animals on choline-deficient diets, the administration of choline caused a marked stimulation of the turnover of liver and plasma phospholipides; this effect is smaller or undetectable in animals maintained on adequate diets. In other words, in experimental animals the stimulating action of a large dose is only evident when the supply of choline or choline precursor is insufficient.

These observations suggest a tentative interpretation of our present findings on human patients. One might postulate that a definite increase in the phospholipide turnover after the administration of a single large dose of choline or methionine reflects a condition of relative deficiency in lipotropic agents. This hypothesis deserves further investigation and we are continuing our study in order to arrive at a more definite conclusion.

In certain instances of chronic hepatitis, the use of lipotropic factors is thought to be of therapeutic value. This beneficial effect is attributed largely to a stimulatory action on the formation of phospholipides. An increase in the turnover of phospholipide in plasma was noted in two patients with portal cirrhosis who received a large dose of choline or methionine. This effect was no longer demonstrable after prolonged treatment with lipotropic agents, although the patients exhibited clinical and laboratory evidence of marked improvement.

It is suggested that the stimulation of phospholipide turnover caused by a single large dose of choline or methionine may indicate a deficiency of lipotropic material, and thus provide an estimate of the anticipated response to the treatment of cirrhosis with lipotropic substances.

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Evidence for the Conversion of Carbon Monoxide to Carbon Dioxide by the Intact Animal

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Conversion of CO to CO_2 by animal tissues was suggested and to some extent demonstrated by Fenn and Cobb (*5*) to account for the marked increases in gas consumption observed when isolated tissues, particularly muscle, were exposed to an atmosphere containing about 80% CO in oxygen. The phenomenon was confirmed by Schmitt and Scott (*9*) and shown to be superimposed on inhibition of the cytochrome-cytochrome oxidase system by Stannard (*10*). The ability of an intact animal to accomplish this conversion, however, has never been demonstrated. In fact, there have been numerous arguments against the presence of such a phenomenon (*6, 7*), the most recent of which is found in the results of Tobias *et al.* (*11*), in which radioactive carbon (as $C^{11}O$) was employed. They found that less than 0.1% of the CO lost from the blood could be recovered as $C^{11}O_2$ in the expired air.

TABLE 1

DISAPPEARANCE OF CO FROM CLOSED CHAMBER (60 LITERS) WITH AND WITHOUT TURTLES*

Exp.	Time†	Total weight of turtles	Initial CO concentration	Amount consumed: With turtles	Amount consumed: Control	Amount consumed: Difference	CO consumed
	days	g	%	ml	ml	ml	%
1	8	2080	0.023	5.92	0.00	5.92	41
2	7	4826	0.020	7.71	1.20	6.51	52
3	13	4880	0.038	12.03	2.68	9.35	48
4	11	4662	0.047	14.86	1.08	13.78	49
5	10	2946	0.075	13.30	0.00	13.30	30

* Gasometric experiments, values expressed at STP.
† Number refers to duration of *each* period, control and experimental.

With the availability of C^{14} and recent refinements in methods for the determination of very small amounts of CO, it was considered of interest to repeat the experiments done in this laboratory with isolated tissue and to extend them to the intact animal. The results with isolated tissue have been reported elsewhere (*3, 4*). Suffice it to say that the oxidation of CO to CO_2 by skeletal and cardiac muscle observed earlier by indirect gasometric and volumetric procedures was quantitatively reproduced in direct experiments measuring conversion of $C^{14}O$ to $C^{14}O_2$.

In the experiments with intact animals reported here, two methods were applied: (a) gasometric measurement of the CO disappearing from a closed chamber, using a modification of the extremely sensitive Roughton-Root technique (*8*), and (b) collection of the expired CO_2 from animals residing in an atmosphere containing CO, some of which was the radioactive $C^{14}O$, and measurement of radioactivity by the method of Bale, *et al.* (*1*).

Table 1 shows the amount of CO lost, measured by the gasometric method, in five experiments with turtles. Expired CO_2 was collected in soda lime placed within the chamber, and oxygen was added automatically as needed from a small spirometer through a check-valve system. Any CO removed for sampling was replaced by introduction of an equal amount from a syringe. Samples were taken initially, after an equilibration period of 10–12 hr and then either daily or at the end of the test period.

[1] Now at Abilene Christian College, Abilene, Texas.